



(19)

Europäisches Patentamt
European Patent Office
Office européen des brevets



(11)

EP 0 346 022 B1

(12)

EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention
of the grant of the patent:
14.02.1996 Bulletin 1996/07

(51) Int Cl.⁶: C07K 7/08, A61K 38/00

(21) Application number: 89305604.4

(22) Date of filing: 02.06.1989

(54) Peptide fragments of HIV

Peptidfragmente von HIV

Fragments peptidiques du VIH

(84) Designated Contracting States:
AT BE CH DE ES FR GR IT LI LU NL SE

- Nixon, Douglas Fraser
Headington, Oxford OX3 0NX (GB)
- Townsend, Alain Robert Michael
Oxford, OX2 6JN (GB)

(30) Priority: 10.06.1988 GB 8813746
05.07.1988 GB 8815956
11.11.1988 GB 8826445

(74) Representative: Matthews, Heather Clare et al
Cambridge CB2 1DP (GB)

(43) Date of publication of application:
13.12.1989 Bulletin 1989/50

(56) References cited:

(73) Proprietor: United Biomedical, Inc.
Hauppauge, New York 11788 (US)

(72) Inventors:
• McMichael, Andrew James
Horton-cum-Studley, Oxford (GB)

- NATURE, vol. 336, 1988; D.F. NIXON et al., pp.
484-487/
- NATURE, vol. 326, 1987; F. GOTCH et al., pp.
881-882/
- NATURE, vol. 328, 1987; B.D. WALKER et al.,
pp. 345-348/
- SCIENCE, vol. 240, 1988; B.D. WALKER et al.,
pp. 64-66/
- PROCEEDINGS OF THE NATL. ACADEMY OF
SCIENCE USA, vol. 85, 1988, Washington, DC
(US); H. TAKAHSHI et al., pp. 3105-3109/

EP 0 346 022 B1

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

Description

This invention concerns peptide fragments of HIV (human immunodeficiency virus) and the use thereof in a potential vaccine against AIDS (acquired immune deficiency syndrome), and for diagnostic and therapeutic purposes.

Background to the Invention

It is known from the prior art (Walker et al., 1987 *Nature* **328**, 345-348) that cytotoxic T lymphocyte (CTL) responses can be generated in humans against the envelope protein (env) of HIV. The same authors suggested some cellular responses to the group association antigen (gag) of HIV were also present in infected individuals, but could not demonstrate that any such response was mediated by CTLs.

Takahashi et al., (1988 *PNAS USA* **85**, 3105-3109) were able to identify a peptide fragment of env which acted as an immunodominant epitope in mice of the H-2^d haplotype. A CTL response to the epitope was demonstrated in such mice, and the authors speculated that the epitope may also act as an epitope in humans, although no evidence was presented in support of this suggestion.

In summary, little was known about CTL responses to HIV in humans, especially in relation to putative CTL responses against gag protein.

Summary of the Invention

In a first aspect the invention provides an in vitro method of stimulating HIV-specific cytotoxic T lymphocytes (CTL), comprising obtaining peripheral blood mononuclear cells (PBMC) from a subject who is seropositive for HIV, incubating one fraction of said PBMC with phytohaemagglutinin for a time sufficient to activate said CTL and thereby produce activated PBMC, washing said activated PBMC, and co-culturing said activated PBMC with a second, unstimulated, fraction of said PBMC for a time sufficient to produce cultured PBMC containing CTL.

Such a method has been found surprisingly effective in the generation of CTL.

In a second aspect the invention provides a method of screening a peptide for an HIV-specific CTL epitope, comprising:

obtaining PBMC from a subject who is seropositive for HIV;
incubating one fraction of said PBMC with phytohaemagglutinin for a time sufficient to activate CTL and thereby producing activated PBMC; washing said activated PBMC; co-culturing said activated PBMC with a second fraction of unstimulated PBMC for a time sufficient to produce cultured PBMC containing CTL; incubating said cultured PBMC with labelled autologous target cells or B lymphoblastoid cells

matched for human leucocyte antigen (HLA) type and said peptide; and determining the amount of label released and thereby identifying an HIV-specific CTL epitope.

5

In a third aspect there is provided a peptide identifiable by the method defined above, the peptide having the amino acid sequence of a fragment of the gag protein of HIV, which interacts specifically with a particular HLA class I molecule to stimulate CTL immunity.

10

One such peptide has the sequence NH₂-lysine-arginine-tryptophan-isoleucine-isoleucine-leucine-glycine-leucine-asparagine-lysine-isoleucine-valine-arginine-methionine-tyrosine-cysteine-COOH, which is derived from the gag (group associated antigen) p24 protein of HIV (i.e. one of the internal core proteins) between residues 263 and 277. The carboxy-terminal cysteine is not part of the gag sequence and is added to facilitate chemical coupling reactions. This peptide interacts specifically with HLA B27, and individuals with HLA B27 (about 7% of the Caucasian population) should respond to the peptide, resulting in production of cytotoxic T lymphocytes (CTL) specific for gag and HLA B27, and capable of lysing cells infected with HIV.

15

Other peptides interact specifically with other HLA molecules and behave in a similar manner on individuals with the relevant HLA.

20

By way of explanation, it has been shown that virus proteins such as gag are presented to T cells as degraded peptide fragments (about 15 amino acids) bound to larger HLA class I molecules on the surface of infected cells. Different peptide regions (epitopes) are recognised and interact specifically with different HLA class I molecules. CTL will only recognise target cells that share HLA Class I molecules, i.e. the T cells recognise a combination of virus antigen plus self HLA. There are probably about 120 different HLA class I molecules, and each individual human has a limited selection, and so will respond only to certain epitopes.

25

Epitopes recognised with different HLA can be identified and isolated in known manner or made by protein synthesis using known techniques.

Peptides in accordance with the invention can be used as the basis of a vaccine against AIDS, by stimulating production of CTL responsive to the relevant HLA and so priming the CTL response.

30

In a further aspect the present invention thus provides a vaccine against AIDS, comprising a peptide identifiable by the method defined above, having the amino acid sequence of a fragment of the gag protein of HIV, which interacts specifically with a particular human leucocyte antigen (HLA) class I molecule to stimulate CTL immunity.

35

The vaccine preferably comprises a peptide which interacts with a relatively common HLA class I molecule, such as HLA A2 which is found in about 40% of the population, or HLA A1, A3, B7 or B44, each of which is found in about 10% of the population.

The vaccine may comprise more than one peptide, and preferably comprises peptides responsive to some of the more common HLA class I molecules to increase effectiveness. For example, a vaccine having peptides responsive to HLA A1, A2, A3, B7 and B44 should stimulate CTL responses in about 90% of the population: the remaining 10% could be protected by herd immunity.

The vaccine may take various different forms. For example, the vaccine may comprise a peptide or mixture of peptides for administration in solution, or absorption onto insoluble material or mixing with an adjuvant. The peptide amino acid sequence could alternatively be used to construct synthetic or fusion proteins that contain the relevant peptide epitopes, by known recombinant DNA techniques. These proteins could be used to immunise as soluble protein or absorbed onto insoluble material or mixed with adjuvant. Alternatively the sequence information could be used to construct recombinant micro-organisms using known techniques which would express the relevant sequences in their own proteins. Examples would be recombinant vaccinia viruses, recombinant poliomyelitis viruses, recombinant BCG, recombinant salmonella, recombinant adenovirus.

Another use of the sequence information would be to construct analogs of the peptides, or other chemicals which would interact with e.g. bind to the HLA molecules involved or the T cell receptors involved and interfere with or stimulate this form of immune response. Inhibition of this type of immunity might be important if this immune response plays a harmful role in any of the pathology caused by HIV. If so it may be important to regulate the levels of this type of T cell immunity in HIV seropositive individuals so as to achieve a balance between beneficial and harmful effects. Stimulation by such agents may be an alternative way of inducing an immune response in seronegative individuals.

The method of screening a peptide for an HIV-specific CTL epitope in accordance with the invention can also be used for diagnostic purposes. In particular, it has been found that it is possible to use a peptide known to comprise such an epitope to identify a T lymphocyte response in a relatively simple assay. Briefly, fresh peripheral blood mononuclear cells (or lymphocytes obtained from biopsy material) are prepared and added at ratios of 50:1, 25:1 and 10:1 to 10^4 51 -chromium labelled B lymphoblastoid cells matched for the relevant HLA molecule. The HLA type of the patient is determined in known manner by tissue typing, and B lymphoblastoid cells obtained from a donor of known HLA type (again determined in known manner by tissue typing) and transformed with Epstein Barr using known techniques. A peptide in accordance with the invention, which interacts with the relevant HLA molecule, is also added to the cells in a concentration of 10 to 100 μ molar. After 4 hours incubation the supernatant is removed and the released 51 -chromium measured. The 51 -chromium released is compared to that released by incubation of labelled cells in detergent, which gives a maximum value,

and that released by labelled cells incubated in medium alone, which gives a minimum value. If lysis (defined by 51 -chromium release of at least two times the minimum value) is observed it means that there are cytotoxic T lymphocytes in the patients' mononuclear cells and these are likely to be indicative of infection with HIV.

Such an assay, together with antibody measurements, may also be useful for measurement of the patient's general immune response to HIV, and may have prognostic implications. This approach may represent a very simple method which can be used for measuring cell mediated immunity in HIV seropositive patients. It may also be possible to automate the method.

By comparing the amount of released label with known standards an indication of the degree of lysis can be obtained and hence of likely infection with HIV.

The in vitro method of stimulating HIV-specific CTLs in accordance with the invention has potential use in therapy. The method involves exposing cultured peripheral blood mononuclear cells, which contain cytotoxic T lymphocytes, to autologous cells, mononuclear cells or B lymphoblastoid cell lines, which have been treated for one hour with an appropriate peptide in accordance with the invention at approximately 10-100 μ g/ml then washed in tissue culture medium and irradiated to 3000 rads. The cells are then cultured in the presence of interleukin-2 at 10 units/ml. Using this method cytotoxic T lymphocyte cell lines specific for the peptide have been grown, and these lines have been expanded up to 10^8 cells. These expanded cytotoxic T lymphocyte lines would be used to treat patients by reinfusion.

Preliminary data indicates that patients with AIDS or the AIDS related complex show low levels of cytotoxic T cell activity, whereas those who are infected with HIV but are healthy show high levels. Part of the immune deficiency syndrome therefore may be a result of impaired cytotoxic T cell activity. The proposal therefore would be to reinfuse autologous cytotoxic T cells grown in vitro on synthetic peptide pulsed cells. Initially patients who had previously had a high cytotoxic T cell activity would be treated at a stage when their levels of these cells was declining. The cytotoxic T cells are preferably derived from lymphocytes taken from the patient at an earlier stage. The lymphocytes may be stored in frozen condition until used for preparation of the cytotoxic T cell line.

The peptide epitope from HIV gag p24, restricted by HLA-B27 is found to be quite well conserved between different strains of HIV 1 and HIV 2, and it was found that cytotoxic T lymphocytes from a patient infected with HIV 1 cross-reacted on the HIV 2 peptide sequence. This means that this peptide and other peptide epitopes from similarly conserved parts of the virus, may be useful in vaccines to stimulate protection against both HIV 1 and HIV 2, and also for diagnostic and therapeutic purposes with patients infected with HIV 1 and/or HIV 2. It is thought that least 50% of such epitopes will be conserved in this way.

The invention will be further described, by way of il-

lustration, in the following example.

Example

Work was carried out on the peptide with the sequence NH₂-lysine-arginine-tryptophanisoleucine-isoleucine-leucine-glycine-leucine-asparagine-lysine-isoleucine-valine-arginine-methionine-tyrosine-cysteine-COOH. The peptide is derived from the gag p24 protein of HIV between residues 263 and 277. The carboxy-terminal cysteine is not part of the HIV gag sequence and was added to facilitate chemical coupling reactions. It may be possible later to define this peptide even more precisely, determining an optimum of 10-12 amino acids.

A way has been worked out of stimulating the circulating precursors of cytotoxic T lymphocytes in peripheral blood to develop into active cytotoxic T lymphocytes, specific for HIV in vitro. This is described in more detail below, but briefly involves stimulating a small fraction of the peripheral blood lymphocytes with the mitogen phytohaemagglutinin (PHA) for 24 hours and then adding these cells to the remaining lymphocytes. The PHA activation probably activates the HIV genome so that HIV viral proteins are synthesized. After culture for seven days, cytotoxic T lymphocyte activity can be measured by killing on autologous target cells (Epstein Barr virus transformed B lymphoblastoid cell lines) that have been infected with recombinant vaccinia viruses that express single HIV proteins.

It has been shown that 70-80 per cent of HIV seropositive donors give a cytotoxic T lymphocyte response to the gag protein, expressed by recombinant vaccinia virus. This cytotoxic T lymphocyte response is HLA restricted which means that when target cells are prepared from unrelated individuals, only those that share HLA class I antigens with the responding T cell are lysed. It has been possible to grow these cytotoxic T lymphocytes in T cell growth factor, which contains the lymphokine Interleukin-2, for up to four weeks.

Having established these HIV gag specific cytotoxic T cell lines, it was possible to test whether they could lyse autologous target cells in the presence of short (up to 20 amino acids) synthetic peptides based on the HIV gag sequence. Previous work has shown that virus proteins such as gag are presented to the T cells as degraded peptide fragments bound to the HLA class I molecules on the surface of infected cells. Following the principles previously discovered and published (Townsend et al., Cell 44:959-968, 1986, McMichael et al., J.Exp.Med. 164: 1397-1406, 1986 and Gotch et al., Nature 326:881-882, 1987) the cytotoxic T lymphocytes, the uninfected target cells and each synthetic peptide were mixed at a final concentration of 10µg/ml. In the presence of the correct peptide the target cells are lysed and gag peptide 263-277 was recognised by cytotoxic T lymphocytes specific for gag and HLA B27. It has been shown previously for influenza virus that there are differ-

ent peptide regions (epitopes) recognised according to the HLA class I molecule involved. For HIV we have defined the above peptide which is associated with the HLA antigen B27. From our previous work with influenza virus we anticipate that all or most individuals with HLA B27 who respond to HIV, will recognise this particular peptide.

This method of finding epitopes can be used to find others that will associate with different HLA class I molecules. The same principle can be applied for any other HIV protein.

Preparation of peripheral blood lymphocytes and induction of HIV specific CTL

40ml venous blood from HIV seropositive donors was placed in heparinised containers (100µl of 100 units/ml preservative free heparin) and was diluted 2:1 with RPMI 1640 tissue culture medium (Gibco) supplemented with 100 units/ml penicillin and 200µg/ml streptomycin, in the absence of serum.

5ml of lymphocyte separation medium (LSM, Flow Laboratories) was carefully underlaid under 15ml of blood/media mixture on a sterile plastic 25ml universal container. The tube was centrifuged at room temperature at 1200 rpm for 30 minutes after which time peripheral blood mononuclear cells (PBMC) were removed from the interface. The PBMC were washed twice with RPMI-1640 then resuspended in 8ml RPMI 1640 + 10% foetal calf serum ("R10") and counted. 1/8th of the PBMC were incubated in 50 ml flasks (Falcon) with added R10 to a concentration of 1.5×10^6 cells/ml. Phytohaemagglutinin (PHA - Wellcome Diagnostics) was added at 1/200 final concentration (2µg/ml). These cells were incubated at 37°C in 5% CO₂ in air for 24 hours. the remaining 7/8th of the PBMC were cultured at a concentration of 1.5×10^6 /ml in R10 at 37°C in 5% CO₂/air in a 50ml tissue culture flask.

After 24 hours the PHA stimulated cells were 40 washed twice in R10, resuspended in R10 at 1.0×10^6 /ml and added to the unstimulated cells. The cultures were left in the incubator for seven days prior to use in the cytotoxic T cell assay. After seven days T cell growth factor (Lymphocult T; Biostest) was added to the culture at 10 units/ml and the growing cells were kept at 0.5×10^6 /ml, being fed with R-10 plus 10µ/ml T cell growth factor every 3 days.

Preparation of target cells

Epstein Barr virus transformed B lymphoblastoid cell lines, autologous matched or mismatched at HLA class I were washed and resuspended to 1.5×10^6 /100µl in R10. 300uCi Na₂⁵¹CrO₄ (Cr⁵¹ Amersham) was added 55 and the cells incubated for one hour at 37°C in 5% CO₂-air. The cells were washed once and then resuspended in peptide solutions (peptide stock dissolved in R10 at 1mg/ml) at a final concentration of 10µg/ml. Each

peptide was tested separately. Each mixture was incubated for one hour at 37°C in 5% CO₂-air, then the cells were washed three times, made up to 2 x 10⁵ cells/ml in R10 and dispensed into microtitre trays (96 well round bottom-Nunc). As controls the autologous B lymphoblastoid cell lines were either uninfected and not peptide treated (negative control) or infected with recombinant vaccinia virus (10pfu/cell for 4 hours at 37°C in 5% CO₂ in air) (positive control) (McMichael et al., J.Gen.Viro 67:719, 1986).

Preparation of effector cells

Cells were harvested by centrifuging at 1500 rpm for 5 minutes and resuspended in R10 for use in the CTL assay.

CTL assay

Target cells were dispensed at 10⁴ cells/well in 96 well round bottomed micro-titre trays in 50µl R10 and effector cells were added in 100µl of the same medium.

Experimental wells were dispensed in duplicate. Controls for background ⁵¹Cr release in the absence of the effector cells (medium controls) and maximum ⁵¹Cr release in the presence of 5% triton ("Triton" is a registered Trade Mark of Union Carbide Chemicals and Plastics Co. Inc.) X-100 (detergent controls) were plated in quadruplicate. Plates were incubated for 4 hours at 37°C, 75µl supernatant was removed into small tubes for counting on the gamma counter. 75µl 10% chloroform was added prior to removal from the containment laboratory. Lysis was calculated from the formula:

$$\frac{\text{medium control} \times 100}{\text{detergent control}-\text{medium control}}$$

Spontaneous ⁵¹Cr release by target cells in medium alone varied between 8% - 30% of count released by triton (registered Trade Mark) X-100.

Claims

1. An in vitro method of stimulating HIV-specific cytotoxic T lymphocytes (CTL), comprising obtaining peripheral blood mononuclear cells (PBMC) from a subject who is seropositive for HIV, incubating one fraction of said PBMC with phytohaemagglutinin for a time sufficient to activate said CTL and thereby produce activated PBMC, washing said activated PBMC, and co-culturing said activated PBMC with a second, unstimulated, fraction of said PBMC for a time sufficient to produce cultured PBMC containing CTL.
2. A method of screening a peptide for an HIV-specific CTL epitope, comprising:

5 obtaining PBMC from a subject who is seropositive for HIV; incubating one fraction of said PBMC with phytohaemagglutinin for a time sufficient to activate CTL and thereby producing activated PBMC; washing said activated PBMC; co-culturing said activated PBMC with a second fraction of unstimulated PBMC for a time sufficient to produce cultured PBMC containing CTL; incubating said cultured PBMC with labelled autologous target cells or B lymphoblastoid cells matched for human leucocyte antigen (HLA) type and said peptide; and determining the amount of label released and thereby identifying an HIV-specific CTL epitope.

- 15 3. A peptide identifiable by the method of claim 2, having the amino acid sequence of a fragment of the gag protein of HIV, which interacts specifically with a particular HLA class I molecule to stimulate CTL immunity.
- 20 4. A peptide according to claim 3, having the sequence NH₂-lysine-arginine-tryptophan-isoleucine-isoleucine-leucine-glycine-leucine-asparagine-lysine-isoleucine-valine-arginine-methioninetyrosine-COOH.
- 25 5. A peptide according to claim 3, having the sequence NH₂-lysine-arginine-tryptophan-isoleucine-isoleucine-leucine-glycine-leucine-asparagine-lysine-isoleucine-valine-arginine-methioninetyrosine-cysteine-COOH.
- 30 6. A method of assaying PBMC of a subject for the presence of HIV-specific CTL, comprising obtaining PBMC from the subject, incubating said PBMC with labelled autologous target cells or B lymphoblastoid cells matched for HLA type and with a peptide in accordance with any one of claims 3, 4 or 5, and determining the amount of label released.
- 35 40 7. A vaccine against AIDS, comprising a peptide according to any one of claims 3, 4 or 5.
- 45 8. A vaccine according to claim 7, comprising a peptide having the sequence NH₂-lysine-arginine-tryptophan-isoleucine-isoleucine-leucine-glycine-leucine-asparagine-lysine-isoleucine-valine-arginine-methionine-tyrosine-COOH.
- 50 9. A vaccine according to claim 7, comprising a peptide having the sequence NH₂-lysine-arginine-tryptophan-isoleucine-isoleucine-leucine-glycine-leucine-asparagine-lysine-isoleucine-valine-arginine-methionine-tyrosine-cysteine-COOH.
- 55 10. A vaccine according to any one of claims 7, 8 or 9, comprising a peptide which interacts with HLA A1, A2, A3, B7 or B44.

11. A vaccine according to any one of claims 7-10 comprising recombinant micro-organism constructed to express the relevant peptide sequence(s).

5

Patentansprüche

1. In vitro-Verfahren zur Stimulierung HIV-spezifischer cytotoxischer T-Lymphocyten (CTL), umfassend den Erhalt peripherer mononukleärer Blutzellen (PBMC) von einem Individuum, das HIV-seropositiv ist, die Inkubation einer Fraktion der PBMC mit Phytohämagglutinin für einen Zeitraum, der ausreichend ist zur Aktivierung der CTL und dabei zur Produktion aktiverter PBMC, das Waschen der aktivierten PBMC und die gemeinsame Züchtung der aktivierten PBMC mit einer zweiten, unstimulierten Fraktion der PBMC für einen Zeitraum, der zur Produktion gezüchterter, CTL-enthaltender PBMC ausreichend ist.
2. Verfahren zum Absuchen eines Peptids nach einem HIV-spezifischen CTL-Epitop, umfassend: den Erhalt von PBMC von einem Individuum, das HIV seropositiv ist; die Inkubation einer Fraktion der PBMC mit Phytohämagglutinin für einen Zeitraum, der ausreichend ist zur Aktivierung von CTL und dabei zur Herstellung aktiverter PBMC; das Waschen der aktivierten PBMC; die gemeinsame Züchtung der aktivierten PBMC mit einer zweiten Fraktion unstimulierter PBMC für einen Zeitraum, der ausreichend ist zur Produktion gezüchterter, CTL-enthaltender PBMC; die Inkubation der gezüchteten PBMC mit markierten autologen Zielzellen oder lymphoblastoiden B-Zellen, die auf einen menschlichen Leukozytenantigen (HLA)-Typ und das Peptid abgestimmt sind; und die Bestimmung der Menge an freigesetztem Marker und die dadurch bedingte Identifizierung eines HIV-spezifischen CTL-Epitops.
3. Peptid, identifizierbar durch das Verfahren nach Anspruch 2, das die Aminosäuresequenz eines Fragments des gag-Proteins von HIV hat, das spezifisch mit einem bestimmten HLA-Klasse I-Molekül zur Stimulierung der CTL-Immunität interagiert.
4. Peptid nach Anspruch 3, das die Sequenz NH₂-Lysin-Arginin-Tryptophan-Isoleucin-Isoleucin-Leucin-Glycin-Leucin-Asparagin-Lysin-Isoleucin-Valin-Arginin-Methionin-Tyrosin-COOH hat.
5. Peptid nach Anspruch 3, das die Sequenz NH₂-Lysin-Arginin-Tryptophan-Isoleucin-Isoleucin-Leucin-Glycin-Leucin-Asparagin-Lysin-Isoleucin-Valin-Arginin-Methionin-Tyrosin-Cystein-COOH hat.

10

6. Verfahren zur Untersuchung von PBMC eines Individuums auf die Gegenwart HIV-spezifischer CTL, umfassend den Erhalt von PBMC von dem Individuum, die Inkubation der PBMC mit markierten autologen Zielzellen oder lymphoblastoiden B-Zellen, die auf einen HLA-Typ abgestimmt sind, und mit einem Peptid gemäß einem der Ansprüche 3, 4 oder 5, und die Bestimmung des Betrages an freigesetztem Marker.
7. Impfstoff gegen AIDS, umfassend ein Peptid nach einem der Ansprüche 3, 4 oder 5.
8. Impfstoff nach Anspruch 7, umfassend ein Peptid, das die Sequenz NH₂-Lysin-Arginin-Tryptophan-Isoleucin-Isoleucin-Leucin-Glycin-Leucin-Asparagin-Lysin-Isoleucin-Valin-Arginin-Methionin-Tyrosin-COOH hat.
9. Impfstoff nach Anspruch 7, umfassend ein Peptid, das die Sequenz NH₂-Lysin-Arginin-Tryptophan-Isoleucin-Isoleucin-Leucin-Glycin-Leucin-Asparagin-Lysin-Isoleucin-Valin-Arginin-Methionin-Tyrosin-Cystein-COOH hat.
10. Impfstoff nach einem der Ansprüche 7, 8 oder 9, umfassend ein Peptid, das mit HLA A1, A2, A3, B7 oder B44 interagiert.

25

11. Impfstoff nach einem der Ansprüche 7 bis 10, umfassend einen rekombinanten Mikroorganismus, der zur Expression der entsprechenden Peptidsequenz(en) hergestellt wurde.

35

Revendications

1. Méthode *in vitro* de stimulation de lymphocytes T cytotoxiques (CTL) spécifiques de VIH, comprenant :

l'obtention de cellules mononucléaires de sang périphérique (PBMC) à partir d'un sujet qui est séropositif pour VIH,
 l'incubation d'une fraction de ces PBMC avec de la phytohémagglutinine pendant une période de temps suffisante pour activer ces CTL et produire ainsi des PBMC activées,
 le lavage de ces PBMC activées,
 et la co-culture de ces PBMC activées avec une seconde fraction non stimulée de ces PBMC pendant une période de temps suffisante pour produire des PBMC cultivées contenant des CTL.
2. Méthode de criblage d'un peptide pour un épitope de CTL spécifique de VIH, comprenant:

- l'obtention de PBMC à partir d'un sujet qui est séropositif pour VIH;
 l'incubation d'une fraction de ces PBMC avec de la phytohémagglutinine pendant une période de temps suffisante pour activer des CTL et produire ainsi des PBMC activées;
 le lavage de ces PBMC activées;
 la co-culture de ces PBMC activées avec une seconde fraction de PBMC non stimulées pendant une période de temps suffisante pour produire des PBMC cultivées contenant des CTL;
 l'incubation de ces PBMC cultivées avec des cellules cibles autologues ou des cellules lymphoblastoïdes B marquées compatibles avec le type d'antigène HLA et ce peptide; et
 la détermination de la quantité de marqueur libérée et ainsi l'identification d'un épitope de CTL spécifique de VIH.
- 5
3. Peptide identifiable par la méthode suivant la revendication 2, ayant la séquence d'acides aminés d'un fragment de la protéine gag de VIH, qui interagit spécifiquement avec une molécule de classe I de HLA particulière pour stimuler une immunité par CTL.
- 20
4. Peptide suivant la revendication 3, ayant la séquence NH₂-lysine-arginine-tryptophane-isoleucine-isoleucine-leucine-glycine-leucine-asparagine-lysine-isoleucine-valine-arginine-méthionine-tyrosine-COOH.
- 30
5. Peptide suivant la revendication 3, ayant la séquence NH₂-lysine-arginine-tryptophane-isoleucine-isoleucine-leucine-glycine-leucine-asparagine-lysine-isoleucine-valine-arginine-méthionine-tyrosine-cystéine-COOH.
- 35
6. Méthode d'essai de PBMC d'un sujet pour déterminer la présence de CTL spécifiques de VIH, comprenant l'obtention de PBMC à partir du sujet, l'incubation de ces PBMC avec des cellules cibles autologues ou des cellules lymphoblastoïdes B marquées compatibles avec le type HLA et avec un peptide suivant l'une quelconque des revendications 3, 4 ou 5 et la détermination de la quantité de marqueur libérée.
- 40
7. Vaccin contre le SIDA, comprenant un peptide suivant l'une quelconque des revendications 3, 4 ou 5.
- 50
8. Vaccin suivant la revendication 7, comprenant un peptide ayant la séquence NH₂-lysine-arginine-tryptophane-isoleucine-isoleucine-leucine-glycine-leucine-asparagine-lysine-isoleucine-valine-arginine-méthionine-tyrosine-COOH.
- 55
9. Vaccin suivant la revendication 7, comprenant un peptide ayant la séquence NH₂-lysine-arginine-tryptophane-isoleucine-isoleucine-leucine-glycine-leucine-asparagine-lysine-isoleucine-valine-arginine-méthionine-tyrosine-cystéine-COOH.